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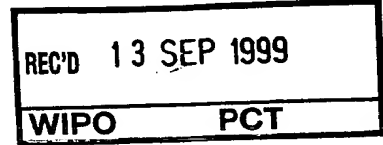
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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 5183 for a patent by PROFESSOR COLIN LESLIE YOUNG filed on 11 August 1998.



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COLIN LESLIE YOUNG

AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

“Durable Mollusc Repellent”

The invention is described in the following statement:

Durable Mollusc Repellent

Field of the Invention

5 The present invention relates to a durable repellent for molluscs. More particularly, the present invention relates to a durable mollusc repellent, which is environmentally-friendly being both non-phytotoxic and harmless to non-target animals, and therefore suitable for use in sustainable agriculture, where the use of toxic chemicals is unacceptable.

10 Background to the Invention

Slugs and snails are major pests of agriculture in many parts of the world. One particular species of slug, the grey field slug, *Deroceras reticulatum*, is a common horticultural and agricultural pest in Australia which causes extensive damage to agricultural crops and garden plants.

15 Significant crop damage by slugs and snails also occurs in the United Kingdom, Northern Europe, the Middle East, North and Central America, South East Asia, Japan and New Zealand. In many cases, the rise to pest status of the slug in question is a consequence of change - either in distribution (as in the case of accidental or deliberate introductions) or in agricultural practice.

In the recent past, fields awaiting seed planting were subjected to a thorough ploughing which actually resulted in the destruction of slug populations submerged beneath the surface of the soil.

20 This maximum tillage of the soil, though, was found to destroy many beneficial characteristics of the soil structure. More recently, in order to reduce expenditure involved in such thorough ploughing and to overcome the difficulties associated with ploughing in muddy, boggy soils, the practice of minimum tillage has increasingly been adopted prior to seed planting. The advent of minimum tillage has contributed significantly to the level of slug populations rising to pest status.

25 Slugs are a major agricultural pest causing significant crop damage because they bury themselves in the soil and then move along into holes drilled for planting new crop seeds in. Once the seed has been placed in the drill holes, they eat the inside out of the new seed, thereby potentially destroying the whole planting. There is therefore an urgent need for a method of protecting seeds from destruction by slugs prior to them being planted.

There are several other instances where a solution to deal with slug populations is required. For example, in order to control weeds and reduce moisture loss by evaporation, it has been common practice to employ sheets of black polythene laid on the ground around the plants and under organic mulching material. The problem associated with such sheets is that they have to be dug up to dispose of them, they are unsightly and they do not allow passage of water through them. Clearly, the use of such material which is non-biodegradable is environmentally unacceptable.

One such solution, which is the subject of Australian Patent No. AU-B-64756/94, has been to employ recycled newspaper waste by reconstituting it into matting, in the form commonly known as "weed mats." Such matting is environmentally friendly since it employs a recycled resource also being one which naturally decomposes. The problem encountered in the use of such matting, however, is that the recycled paper mat is attacked by slugs in the soil upon which the matting is laid. This attack reduces the effective lifespan of the matting thereby making such a slug barrier unattractive to consumers. In addition, the laying of such matting would be very labour-intensive and time-consuming and it would be preferable if the time interval between successive applications could be extended as much as possible. None of the molluscicides presently available on the market would be suitable for this application since their efficacy reduces rapidly upon exposure to rain or moisture and so would be ineffective in extending the lifespan of the weed mats.

Another case where slugs present a problem is in the agricultural practice of growing brussel sprouts. These crops present one of the few instances where slugs actually move up onto the plant and shelter in the plant itself, rather than stay in the soil and attack the root system. The problem presented in this case is that a slug repellent is required which is non-phytotoxic as well as being harmless to human beings, the eventual consumers of the crop. A further problem is that the slug repellent cannot be in the form of a bait formulation, but rather in the form of a spray since the repellent has to coat the leaves of the plant. Preferably, the slugs must be prevented from climbing up onto the plants at a very early stage because once they have done so it is very difficult to remove them. It has been proposed that growers confronted with this problem may use a surfactant repellent such as CeTAB, cetyl tertiary ammonium bromide. However, the efficacy of this repellent is short-lived, since the compound is soluble and washes off in the rain.

As was also mentioned above, one of the ways in which mollusc populations can achieve pest status is that they can be accidentally introduced. On such mode of accidental introduction is where molluscs attach themselves onto the hulls of ships which move from port to port. One way in which such accidental introductions could be prevented is for a mollusc repellent to be applied to the ship's hull thereby preventing the initial harbouring of the mollusc. In the United States, a considerable problem has been encountered in the control of the introduced *Zebra mussel*, which attaches itself to the inlet and outlet pipes of cooling systems for industrial power generators. This mollusc has now reached plague proportions. To solve the mollusc problems encountered in these two aqueous situations, care would also have to be taken to apply a mollusc repellent that was not soluble and one that would not endanger aquatic life. None of the known mollusc repellents would be useful in this application all being either too soluble or too toxic.

Chemical methods (*i.e.* the use of molluscicides) involving the use of stomach poisons for the control of these pests are well known. Molluscicides containing metaldehyde and methiocarb have been in use for some while, but these are themselves toxic to non-target animals and human beings and in bait form, they also deteriorate upon exposure to rain and are not sufficiently durable as long-term repellents.

The use of metal complexes in molluscicides was first proposed by Henderson *et al.* in "Aluminium(III) and Iron(III) complexes exhibiting molluscicidal activity," Australian Patent AU-B-22526/88. In one of their studies, these workers compared the relative toxicities of some aluminium and iron salts and chelates and their efficacies as stomach poisons by injecting known amounts into the gut lumen of molluscs and they found that, in fact, the metal chelates were more toxic than their corresponding salts. Metal chelates were also first trialed by Henderson *et al.* as contact-action poisons. In one particular study, Henderson used the metal chelate, FeEDTA, as the toxic agent, finding it just as effective as various salts of Fe(III). (Henderson, I.F. *et al.*, in "A New Group of Molluscicidal Compounds," **BCPC mono.**, (1989), **41**, "Slugs and Snails in World Agriculture", pp 289-294 eds. Henderson, I.F., British Protection Council, Farnham, U.K.).

More recently, Puritch *et al.* in "Ingestible Mollusc Poisons," International Patent Application No. WO 96/05728 have claimed a terrestrial mollusc stomach poison containing as the active ingredient either ferric edetate or the ferric hydroxy-ethyl derivative of edetic acid. These

workers have also shown that mixtures of iron salts such as ferric sulphate, ferric chloride or ferric nitrate when mixed together with disodium EDTA or EDTA as such are toxic to the slug species, *Deroceras reticulatum*. The present inventor has also developed a stomach-action molluscicide, (see International Patent Application No: PCT/AU97/00033) containing the oxo-dimer, [EDTA-Fe- μ -Fe-EDTA]⁺ as the active ingredient which was found to be more palatable to molluscs and therefore also more efficacious. While metal chelate-derived molluscicides are preferable to the use of the more toxic alternatives, such molluscicides are all soluble and are therefore not applicable to any of the problems outlined above.

Dawson *et al*, in **BCPC Symposium Proceedings**, (1996), 66, "Slug & Snail Pests in Agriculture," p 439-444, eds. Henderson, I.F., British Protection Council, Farnham, U.K.). have investigated the repellency of a range of surfactants to the slug, *Deroceras reticulatum*. They found that in particular, tetraammonium salts were highly repellent and some polyphenylpolyethoxylates were also repellent with the degree of repellency varying with the degree of ethoxylation. These workers were interested in using surfactants as a repellent to crops.

Their laboratory tests showed that crawling slugs rapidly detect and are deterred by topical applications of chemicals at low deposit rates. They concluded however, that surfactants were of limited use in this type of application, since they were rapidly removed from the plant by rain and by condensation.

Mollusc repellent formulations commonly in use contain methiocarb, copper sulphate, aluminium sulphate, ammonium alum and thiram to mention a few. Aluminium sulphate and ammonium alum work as taste repellents, whilst methiocarb and thiram are toxic poisons. Copper sulphate and copper oxychloride have been used for some time as slug repellents but they are too soluble to be effective as a slug repellents for the above-mentioned applications seed and moreover both have a low LD₅₀ value (see Table 1) by virtue of them being soluble and therefore being able to enter the blood stream by biochemical degradation. In addition, they are both phytotoxic.

Copper silicate, which is the subject of Australian Patent No. AU-B-27621/92, is also believed to function as a fungicide and a pesticide. Although this compound is not phytotoxic, it is too soluble to be able to function effectively as the active ingredient in a durable slug repellent composition for a seed coating or for any of the applications mentioned above.

Accordingly, it is an object of the present invention to provide a mollusc repellent which achieves the requirement of repellency together with the requirement of durability. It is also an object of the present invention to provide a durable mollusc repellent, which is environmentally-friendly being both non-phytotoxic and harmless to non-target animals, and therefore suitable for use in sustainable agriculture, where the use of toxic chemicals is unacceptable.

Summary of the Invention

The present study concentrated on finding an active ingredient, for both horticultural and agricultural purposes, that acted both as a repellent to molluscs and one that remained efficacious in field conditions when it rained and was effective in both marine and fresh-water environments. The results of trials conducted on several metal complexes revealed that some metal oxalates, and in particular, copper oxalate, fulfilled these requirements.

In addition, the acute toxicity of copper oxalate is not known, but it is estimated that the toxicity is low (*i.e.* greater than 1000 mg/kg of body weight). This estimation is based on the following reasoning. Copper oxalate has extremely low solubility in water over a considerable range of pH. It is thought that only a miniscual amount of copper oxalate will be absorbed in the mammalian gut. The toxicity of a range of copper compounds is known and these are summarised in Table 1. It is apparent that compounds with low solubility are not very toxic. Oxalates are known to be toxic and some data are given in Table 1. Oxalates occur in a wide range of natural products including some foods, such as tea, wheat, spinach and rhubarb. Actual poisonings by oxalate are known to arise more from natural sources than from synthetic materials. It appears that the toxicity of oxalates is due to the fact that they chelate metals that are needed for biochemical/metabolic processes.

Copper oxalate itself occurs naturally in the soil due to the decomposition of organic matter. The fact that copper oxalate is very insoluble seems to be employed by certain common soil fungi, *Aspergillus niger*., which has been found to solubilize a number of minerals including cuprite, CuO₂, with no reduction in growth rate. (Sayer, J.A., Kierans, M. and Gadd, G.M., "Solubilisation of some Naturally Occurring Metal-bearing Minerals, Limescale and Lead Phosphate by *Aspergillus niger*," **FEMS Microbiology Letters**, (1997), 154, 29-35, published by Elsevier).

After one to two day's growth on cuprite at 25°C, a precipitate of copper oxalate was observed. It was suggested that this oxalate formation represented a reduction in bioavailability of toxic cations and represented an important means of toxic metal immobilisation of physiological and environmental significance. A certain amount of copper is, of course essential for both plant and animal life, it being an important co-enzyme in a number of biochemical reactions. The average daily requirement for man is estimated to be 2-5 mg per day (Henry Osiecki, "Nutrients in Profile," (1995), published by Bioconcepts Pty Ltd).

The toxicity of copper oxalate to plants is very low. In published results, Crosier *et al* (Crosier, W., Nash, G. and Crosier, D.C., "A Dry Non-phytotoxic Bird and Insect Repellent," **Proc. Ass. Off. Seed Analysis**, (1970), **60**, 206-212) investigated the effect of copper oxalate on wheat seeds and failed to detect any reduction in germination percentages.

Copper oxalate is registered in the United States as a bird repellent and the belief is widely held that its efficacy might largely be due to birds being averted from food treated with copper oxalate by sight due to its blue colour. Copper oxalate in one study, however, was found to be ineffective on free-ranging birds (Conover, M.R., "Response of Birds to Different Types of Food Repellents," **Journal of Applied Ecology**, (1984), **21**, 437-443), so its ability to act as a bird effective repellent was not conclusive. In addition, there are many chemicals which are used as a deterrent against birds that would be ineffective against slug populations for the requirements of the present application, such as aluminium sulphate and ammonium alum, for example.

According to one aspect of the present invention, there is provided a durable mollusc repellent composition including an effective amount of an insoluble metal oxalate and a suitable carrier therefor.

Preferably, the metal of the metal oxalate is selected from a transition metal or a transition metal in combination with a non-transition metal. More preferably, the metal is selected from the group of iron(II) or iron(III), aluminium, zinc or copper. Most preferably, the metal is copper. Preferably, the non-transition metal is potassium. Preferably, the metal oxalate is selected from ferric potassium oxalate or copper oxalate. Most preferably, the metal oxalate is copper oxalate.

Typically, the amount of metal oxalate required for efficacy is between about 5% to 100% by weight of the total composition. Preferably, the amount is about 2-10% by weight of the total composition. Most preferably, the amount is about 5% of the total composition of the repellent.

5 According to another aspect of the invention, there is provided a method of treating an article with the mollusc repellent, which is the subject of the invention, comprising applying the composition to the surface of the article to be treated. Preferably, the article includes edible and non-edible articles. Most preferably, the edible article is selected from seeds, including wheat, barley, grass (clover, phalaris, rye and cocksfoot), canola seeds, fruit or vegetables. Most preferably, the non-
10 edible article is selected from weed mats, inlet and outlet pipes for cooling systems, hulls of ships, drive-ways of homes and grow-bags, but is not limited to these.

In a preferred embodiment, the repellent composition further includes a carrier. Preferably, the carrier is water, wherein the metal oxalate forms an aqueous suspension. Preferably, the carrier comprises between about 0-95% by weight of the total composition.

15 In a preferred form of the invention, the repellent composition further includes an edible binder which facilitates the adhesion of the metal oxalate onto the surface of the article to be treated. Preferably, the binder is selected from gum arabic or gum acacia where the repellent is to be applied to edible articles. Preferably, the binder is selected from a waterproof binder such as paraffin wax, white oil, casein or polyvinylacrylamide where the repellent is applied to non-edible articles. Preferably, the binder comprises between about 0.1-5% by weight of the total
20 composition. Most preferably, the binder comprises 0.5-3% by weight of the total composition of the repellent.

Preferably, where the treated article is a seed having the potential to produce at least one root/s, the composition further includes a growth hormone such that the hormone is readily available to the root/s as it/they emerge/s from the seed. Preferably, the growth hormone is a seaweed extract.
25 Preferably, the growth hormone comprises between about 0.05% and 1% by weight of the total composition of the repellent.

Preferably further, where the treated article is a seed, the composition further includes a fungicide such that the emerging root/s is/are protected from attack by a fungus. Preferably, the fungicide is selected from copper oxychloride or thiram. Preferably, the fungicide comprises about 0.05% to

1.0% by weight of the total composition of the repellent. Typically, the fungicide is applied as a thin coating in combination with a small amount of a non-phytotoxic dye, the latter being used as an indicator that the seeds have been treated with a fungicide.

5 Preferably, the non-phytotoxic dye comprises less than about 1% of the total composition of the repellent.

Preferably, the carrier further comprises a diluent to ensure even coverage of the article to which the repellent is to be applied. Preferably, the diluent is selected from a silicate, gypsum or limestone. Preferably, the diluent comprises between about 0 % to 95% of the total composition.

10 According to yet another aspect of the invention, the metal oxalate comprises a metal oxalate in combination with at least one other mollusc repellent.

The mollusc repellent is advantageously presented in a solid form such as tablets, powders, granules, as a suspension in water or as a paint, dipping or coating composition. Those skilled in the art will appreciate that it is preferable to prepare the products which are the subject of the invention in a form that is easy for consumers to use. Tablets, powders or granules can be
15 sprinkled over the ground or in letter boxes to repel snails and slugs. An aqueous suspension of metal oxalate composition as detailed above can be used for submersing seeds in, for spraying on driveways, for spraying on the trunks of fruit trees or for spraying on vegetable seedlings, such as brussels sprouts. A repellent composition can be applied either by sprinkling on the surface of, in solid form, or by painting on the surface of, in the form of a paint composition, the lining of grow-
20 bags, used by the home gardener, or at least one of the layers that comprise "weed mats", as described above. A repellent composition in the form of a marine paint, can be applied to inlet and outlet pipes for cooling systems or on the hulls of ships to prevent the introduction of foreign species of unwanted molluscs from other parts of the world.

The mollusc repellent may also conveniently be presented as a two-part composition, wherein the
25 first part comprises an effective amount of an aqueous solution of oxalic acid or soluble oxalate and the second part comprises an effective amount of an aqueous solution of a soluble metal salt, whereby sequential application of the two solutions, in either order, results in the in-situ preparation of a metal oxalate. Preferably, the metal oxalate is selected from ferrous oxalate, ferric ammonium/potassium oxalate or copper oxalate. Preferably, the oxalic acid and the metal

salt are present in equimolar amounts. Preferably further, the concentration of the metal salt solution is about 5%. Such use of two environmentally acceptable chemicals renders such a mollusc repellent suitable for sustainable agriculture purposes.

According to another aspect of the invention, the invention provides an article which has been treated with the mollusc repellent composition. Preferably, the treated article is selected from a seed, a weed mat, a citrus tree, a driveway, an inlet or outlet pipe, a ship's hull, a grow-bag, a vegetable seedling, but is not limited to one of these.

Throughout this specification, unless the context requires otherwise, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not to the exclusion of any other integer or group of integers.

Examples

The oral toxicity of several soluble copper compounds and oxalates which have been extracted from "SAX'S Dangerous Properties of Industrial Materials," edited by Lewis, R.J., are given in Table 1. LD50 values have generally been given for the rat since these were available for all the chemicals of interest in this study. However, where available, LD50 values for the human being have also been given.

Table 1. Oral Toxicity of several soluble copper compounds and oxalates

Chemical Compound	LD50 (Rat) mg/kg	LD50(Human) mg/kg
Copper acetate	595	
Copper oxychloride	700	
Copper citrate	1580	
Copper nitrate	940	
Copper sulphate	300	50
Copper hydroxide		200
Oxalic acid	7500	
Potassium oxalate	660	

The results in Table 1 show that several copper compounds have shown quite considerable toxicity to the rat and to the human being, whereas because copper oxalate is insoluble, its LD50 values both for the rat and the human being would be expected to be large.

5

The invention will now be illustrated with reference to the following non-limiting Examples.

Example 1.

- 10 There are many possible variables to consider when evaluating snail or slug repellents. Field trials are often poorly controlled and it is often difficult to arrive at unambiguous conclusions. It is impossible to apply extensive statistical analysis to poorly designed or controlled experiments. However, a series of simple experiments in which variables are controlled lead to unambiguous conclusions with no need for statistical analysis. It was decided to compare the repellents under
15 laboratory conditions which could closely mimic controlled field conditions.

Repellency of the test compounds was tested using the sectorised filter technique of Bowen and Antoine (*see* Bowen, I.D. and Antoine, S., "Molluscicide Formulation Studies," **International Journal of Pest Management**, (1995), 41(2), 74-78.)

The compounds tested were copper oxalate and CeTAB (cetyl tertiary ammonium bromide).

- 20 One sector of the filter paper was used as the control and this was moistened with distilled water (1 ml). The other half was moistened with the test compound. The copper oxalate used in the trial was insoluble and so was rubbed into the filter paper and the weight of the solid held on the filter paper was calculated. The paper containing the copper oxalate was moistened with water after the addition of the chemical.
- 25 Filter sectors were placed on the lid of a plastic tub. The tub was inverted and was used to make a closed test arena. A small gap of 2 mm was left between the two filter sectors so as to prevent mixing of the test paper with the control. One juvenile *Helix aspersa* was introduced to the control sector and was allowed to remain in the arena overnight.

Attractancy / repellency was detected by dusting the filter paper with charcoal to reveal the slug trails. The results were quantified by placing a transparent grid over the test sectors and measuring the area covered by snail trails. Any phagostimulatory effect of the test compound was indicated by the consumption of the treated paper.

5

Results

The results of the arena trials including the final position of each snail at the end of the experiment, the percentage of the filter paper section eaten and the percentage trail cover are shown in Table 2.

10 **Table 2. Results of attractancy / repellency tests on Copper oxalate and CeTAB**

Treatment	Concentration (mg/cm ²)	Final Position	% Sector Eaten		% Covered	
			Test	Control	Test	Control
Control		C	5	3	84	88
		C	5	1	88	80
		T	0	5	88	77
CeTAB	0.2	R	0	2	20	86.7
	0.2	R	0	4	22	77.8
	0.2	R	0	1	42.2	75.6
	2.0	R	0	4	0	62.2
	2.0	R	0	5	0	66.6
	2.0	R	0	3	0	71.1
	2.0	R	0	5	0	71.1
Copper oxalate	2.11	R	0	5	0	71.1
	1.61	R	0	4	4.4	88
	2.43	R	0	2	0	68.9
	2.47	R	0	5	0	86.7
	3.46	R	0	1	4.4	66.7
	3.19	C	0	2	0	88

where R = roof of the container; T = test sector; and C = control sector.

Summary of Results

The results show that snails were strongly repelled by the copper oxalate. In all, of the six snails tested, none were found in the test sector at the end of the experiment and there was no consumption of the test filter paper. There was very little mucous cover compared to the control.

- 5 The 'positive control, CeTAB, was also found to act as a repellent. Indeed, there was a concentration-related response, more snails being repelled by 2.0 mg/l CeTAB, compared to 0.2 mg/l CeTAB.

Example 2.

10 In the following experiment, various seeds were coated with copper oxalate and Ferric potassium oxalate, respectively and fed to the slug, *Deroceras reticulatum*. The damage to these seeds was compared to damage to control seeds which were left untreated. The percentage of seeds that were damaged was calculated and is given in Table 3.

15 Table 3. Protection afforded to seeds by Copper oxalate and Ferric potassium oxalate

Treatment	% Seeds damaged		% Seeds damaged		% Seeds damaged	
	Wheat seeds		Pea seeds		Broad beans	
	After 5 days	7 days	After 5 days	7 days	After 7 days	
Control	37	87	15	33	5	
Copper oxalate	0	2	7	7	5	
Ferric potassium Oxalate	3	20	15	17	0	

Summary of Results

It can be seen from the above results that copper oxalate is a very effective slug repellent for wheat and pea seeds.

Example 3.

In the following experiment, the efficacy of copper oxalate for the protection of seeds was tested and compared to **BAYSOL** and **MULTIGUARD**. The results are shown in Table 4. Seeds
 5 were coated with an aqueous suspension of copper oxalate, dried and fed to the slug, *Deroceras reticulatum*.

Table 4. Protection afforded to wheat and pea seeds by copper oxalate compared to BAYSOL and MULTIGUARD

Treatment	<u>% Seeds damaged</u>	<u>% Seeds damaged</u>
	Wheat seeds	Pea seeds
	After 9 days	After 7 days
Control	72	23
Copper oxalate	10	8
MULTIGUARD		2
BAYSOL	10	4

10 **MULTIGUARD** and **BAYSOL** are registered trade marks of Multicrop (Aust.) Pty Ltd and Bayer, respectively.

Summary of Results

The above results show that copper oxalate dramatically reduces the amount of damage to wheat
 15 seeds and does so to the same extent as **BAYSOL**, a toxic molluscicide containing methiocarb. Copper oxalate was also found to be effective at protecting pea seeds from damage by slugs although not as effectively as **MULTIGUARD** which was more effective than **BAYSOL**. These latter two products also resulted in the death of the slugs involved in the trial.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification (unless
5 specifically excluded) individually or collectively, and any and all combinations of any two or more of said steps or features.

COLIN LESLIE YOUNG

7 August 1998

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